DATA EVALUATION RECORD

CAPHRA/λ-CYHALOTHRIN

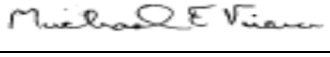
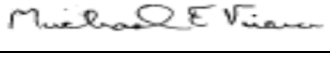
Study Type: OCSPP Non-Guideline; *In Vitro* Metabolism Kinetics

EPA Contract No. EP-W-16-018
Task Assignment No.: 32-3-034 (MRID 50803905)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

3201 Jermantown Rd., Suite 400
Fairfax, VA 22030

Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 05/20/2019
Secondary Reviewer: Michael E. Viana, Ph.D., DABT	Signature: Date:	 05/24/2019
Quality Assurance: Sarah E. Saucier, Ph.D.	Signature: Date:	 05/31/2019
Project Manager: Michael E. Viana, Ph.D., DABT	Signature: Date:	 05/31/2019

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel**.** Contractor's role did not include establishing Agency policy.

EPA Reviewer: Evisabel Craig, Ph.D., DABT **Signature:** E.C. Craig
Risk Assessment Branch VI, HED (7509C) **Date:** 8/1/2019
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DATA EVALUATION RECORD

STUDY TYPE: OCSPP Non-guideline; *In Vitro* Metabolism Kinetics.

PC CODE: 128897

DP BARCODE: D451266

TXR#: 0057896

TEST MATERIAL (PURITY): λ-Cyhalothrin (98.1% a.i.)

SYNONYMS: (*R*)-cyano(3-phenoxyphenyl)methyl (1*S*,3*S*)-*rel*-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate

CITATION: Brown, S. (2019) λ-Cyhalothrin: A study to determine the kinetics of metabolism of λ-cyhalothrin in selected expressed human carboxylesterase (CES) and cytochrome P450 (CYP) enzymes; final report. Concept Life Sciences Dundee, Dundee Technopole, Dundee, United Kingdom. Laboratory Project ID: CXR1723-V λ-cyhalothrin, January 10, 2019. MRID 50803905. Unpublished.

SPONSOR: Council for Advancement of Pyrethroid Human Risk Assessment, LLC (CAPHRA), c/o Household and Commercial Products Association, 1667 K Street, NW, Suite 300, Washington DC

EXECUTIVE SUMMARY: In a non-guideline, *in vitro* metabolism study (MRID 50803905), the apparent intrinsic clearance (CL_{int}) of λ-cyhalothrin (98.1% a.i.; Batch # 647118) was determined in recombinant microsomes. The enzymes expressed by these systems were CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP2D6*1, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CES1b, and CES2 (see Appendix 1 at the end of this DER for product details); a control system also was included. For the CYP enzymes, preliminary experiments were conducted with λ-cyhalothrin to screen for metabolism by the enzyme variants. Duplicate incubations were conducted with the CYP enzymes (10 pmol/mL) and λ-cyhalothrin (0.1 µM) with a minimum protein concentration of 0.1 mg/mL achieved by the addition of control microsomes. Incubations with control microsomes at the same concentrations as the CYP microsomes were performed to correct for endogenous CES activity. If the total rate (k_{dep}) for the CYP enzyme was greater than twice the rate of the control microsomes, it was selected for further experimentation. For the CES enzymes, a preliminary experiment was conducted with duplicate incubations of λ-cyhalothrin (0.1 µM) with CES1b at 2, 5, and 10 pmol/mL and CES2 at 2, 5, and 20 pmol/mL with bovine serum albumin (BSA) to achieve a minimum protein concentration of 0.1 mg/mL. Control incubations also were performed with BSA alone to account for non-enzymatic loss of λ-cyhalothrin. A second preliminary experiment was conducted for CES2 at 20, 50 and 100 pmol/mL because no metabolism was

observed at ≤20 pmol/mL. The main study was conducted in duplicate with CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP3A4, CYP3A5, CES1b, and CES2 in the presence of NADPH (CYP only) with 0.1 µM λ-cyhalothrin. Two experiments were conducted with the data from the first experiment used for selection of time points used in the second experiment (a third confirmatory experiment was conducted for CYP2C19). The final concentrations of λ-cyhalothrin were determined by LC/MS.

Rates of depletion were determined from plots of the natural logarithm of the percentage of λ-cyhalothrin remaining against incubation time by linear regression. Estimates of CL_{int} for CYP and CES enzymes were calculated with the following equation:

$$CL_{int} = \frac{0.693}{t½} \times \frac{\text{Incubation volume (mL)}}{\text{pmol CYP or CES}}$$

RESULTS

PRELIMINARY EXPERIMENTS: Duplicate incubations with 0.1 µM λ-cyhalothrin in recombinant human microsomes were conducted in the presence of NADPH; incubation times were not reported. Preliminary results for the CYP enzymes were presented in Table 1 on page 20 of MRID 50803905 and are included in Appendix 2 at the end of this DER. CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP3A4, and CYP3A5 metabolized λ-cyhalothrin at ≥1.63× the rate of control microsomes and were selected for the main study experiments. The increases for CYP2C8, CYP2C9*1, and CYP3A5 did not achieve the two-fold criterion level but were selected regardless. Preliminary results for the CES enzymes were presented in Table 2 on page 21 of MRID 50803905 and are included in Appendix 2 at the end of this DER. A concentration of 2 pmol/mL was selected for CES1b for the main study. Because no metabolism was observed at any CES2 enzyme concentration (2-100 pmol/mL), a concentration of 200 pmol/mL CES2 with a 2-h incubation time was selected for the main study.

MAIN STUDY EXPERIMENTS: Results for the main study experiments were presented in Tables 3 (CYP) and 4 (CES) on pages 22 and 23,respectively, of MRID 50803905 and are included in Appendix 3 at the end of this DER. Because there was a three-fold difference in calculated CL_{int} values for metabolism in CYP2C19 between the experiments carried out on May 10 and June 8, a further experiment was conducted on June 14 with two separately prepared λ-cyhalothrin solutions and increased incubation times (not specified). The data from the third experiment (June 14) were similar to the data from May 10; therefore, the data from June 8 were not used in the calculation of the mean values.

Because there was a 1.7-fold difference in calculated CL_{int} values for metabolism in CES2 between the experiments conducted on May 29 and June 4, a further experiment was conducted on June 14. The mean calculated CL_{int} from the three experiments was reported; however, the percentage loss was <20% over the 2-h incubation period.

λ-Cyhalothrin was metabolized by six of the expressed CYP enzymes (CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP3A4, and CYP3A5) with CYP2C19 having the highest estimated CL_{int}. Estimated CL_{int} values ranged from 0.248 µL/min/pmol for CYP2C8 to 4.76 µL/min/pmol

for CYP2C19. λ-cyhalothrin was metabolized to a generally lesser extent by CES1b and CES2 with estimated CL_{int} values of 0.935 µL/min/pmol and 0.00327 µL/min/pmol, respectively.

REVIEWER'S COMMENTS: This is a non-guideline study and was submitted as part of CAPHRA's effort to assess the pharmacokinetic properties of the pyrethroids.

APPENDIX 1

Source of test systems: Recombinant human "supersomes" were purchased from Corning B.V. Life Sciences, Amsterdam, The Netherlands. These "supersomes" are microsomes having recombinant human enzymes expressed by baculovirus-infected insect cells (see table below).

Expressed enzyme	Product number	Lot Number	Expressed enzyme	Product number	Lot Number
CYP1A2	456203	7320001	CYP3A4	456202	5224002
CYP2A6	456254	5315001	CYP3A5	456256	5258001
CYP2B6	456255	5239002	CYP3A7	456237	5246004
CYP2C8	456252	7255002	CYP4A11	456221	5266001
CYP2C9*1	456258	6257001	CES1b	453320	6230004
CYP2C19	456259	7262001	CES2	453322	6084004
CYP2D6*1	456217	456217	Control	456244	6180001
CYP2E1	456206	5265003			

(copied from page 11 of MRID 50803905)

APPENDIX 2

Expressed enzyme	Date	λ-cyhalothrin conc µM	Total K_{dep} min^{-1}	Control K_{dep} min^{-1}
CYP2C19	04-Apr-18	0.1	0.0459	0.00420
CYP3A4	05-Apr-18	0.1	0.0186	0.00380
CYP2B6	04-Apr-18	0.1	0.0135	0.00380
CYP3A5	05-Apr-18	0.1	0.00810	0.00440
CYP2C9*1	04-Apr-18	0.1	0.00660	0.00380
CYP2C8	04-Apr-18	0.1	0.00621	0.00380
CYP2D6*1	05-Apr-18	0.1	0.00525	0.00380
CYP4A11	05-Apr-18	0.1	0.00416	0.00360
CYP2A6	04-Apr-18	0.1	0.00351	0.00380
CYP2E1	05-Apr-18	0.1	0.00315	0.00380
CYP3A7	05-Apr-18	0.1	0.00286	0.00380
CYP1A2	04-Apr-18	0.1	0.00264	0.00380

(copied from page 20 of MRID 50803905)

Expressed enzyme	Date	λ-cyhalothrin conc µM	CES K_{dep} min^{-1}
CES1b 2 pmol/mL	11-Apr-18	0.1	0.00273
CES1b 5 pmol/mL	11-Apr-18	0.1	0.00137
CES1b 10 pmol/mL	11-Apr-18	0.1	0.00539
CES2 2 pmol/mL	11-Apr-18	0.1	No metabolism detected
CES2 5 pmol/mL	11-Apr-18	0.1	No metabolism detected
CES2 20 pmol/mL	11-Apr-18	0.1	No metabolism detected
CES2 20 pmol/mL	04-May-18	0.1	No metabolism detected
CES2 50 pmol/mL	04-May-18	0.1	No metabolism detected
CES2 100 pmol/mL	04-May-18	0.1	No metabolism detected

(copied from page 21 of MRID 50803905)

APPENDIX 3

Expressed Enzyme	Date	λ-Cyhalothrin Conc µM	kdep min^{-1}	$t_{1/2}$ min	CL_{int} µL/min/pmol CYP	Mean CL_{int} µL/min/pmol CYP
[1]CYP2C19	10-May-18	0.1	0.0588	11.8	5.88	4.76
	08-Jun-18	0.1	0.0196	35.3	1.96	
	14-Jun-18	0.1	0.0306	22.6	3.06	
	14-Jun-18	0.1	0.0534	13.0	5.34	
CYP3A4	10-May-18	0.1	0.0137	50.8	1.37	1.54
	08-Jun-18	0.1	0.0172	40.4	1.72	
CYP2B6	10-May-18	0.1	0.0173	40.0	1.73	1.44
	08-Jun-18	0.1	0.0115	60.3	1.15	
CYP3A5	10-May-18	0.1	0.00284	244.0	0.284	0.309
	08-Jun-18	0.1	0.00334	207.9	0.334	
CYP2C9*1	10-May-18	0.1	0.0021	324.2	0.214	0.257
	08-Jun-18	0.1	0.0030	231.8	0.299	
CYP2C8	10-May-18	0.1	0.0021	336.3	0.206	0.248
	08-Jun-18	0.1	0.0029	238.5	0.291	

[1] Data from the CYP2C19 experiment on 08 June 2018 should not be used, and have not been included in the calculation of means, as there is a notable difference in calculated CL_{int} between this experiment and those of 10 May 2018 and 14 June 2018.

Rates of λ-cyhalothrin depletion in control supersomes were subtracted from the total rate to correct for endogenous CES activity.

(copied from page 22 of MRID 50803905)

Expressed Enzyme	Date	λ-Cyhalothrin Conc µM	kdep min^{-1}	$t_{1/2}$ min	CL_{int} µL/min/pmol CES	Mean CL_{int} µL/min/pmol CES
CES1b 2 pmol/mL	29-May-18	0.1	0.00217	319.4	1.086	0.935
	04-Jun-18	0.1	0.00157	442.4	0.784	
CES2 200 pmol/mL	29-May-18	0.1	0.000365	1898.2	0.00183	0.00327
	04-Jun-18	0.1	0.000614	1128.6	0.00307	
	14-Jun-18	0.1	0.000983	705.7	0.00491	

Rates of λ-cyhalothrin depletion in BSA were subtracted from the total rate to correct for non specific loss of λ-cyhalothrin.

The percentage loss of λ-cyhalothrin was less than 20% over 2 hours in CES2 at 200 pmol/mL. Mean CL_{int} from 3 experiments has been reported.

(copied from page 23 of MRID 50803905)